Studiul comparativ al infestaţiei cu cyathostome a cailor din Portugalia şi România pe baza identificării subpopulaţiei de L3 de *Cyathostomum sensu latum*

Comparative study of cyathostomin horse infection in Portugal and Romania based in L3 subpopulations of *Cyathostomum sensu latum*

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Abstract

A comparative study of prevalence/abundance and analysis of different morphological types of *Cyathostomum sensu latum* L3 was performed in faecal samples of domestic and feral horses and donkeys in Portugal and Romania, from 2000 till 2008.

Eight morphological types of *Cyathostomum sensu lato* L3 were recorded: five with 8 cells (A, B, C, D and G) and other three with 6, 7 and 9 cells (E, F and H). The most abundant in Portugal belonged to *Cyathostomum* type A (65-98%), followed by type D (3,9-9,7) and C (2,3-20,6). The larval types with lower abundance were B (1,4-2,2) and E to H (0,3-3,2), rarely referred in the literature. Type A L3 was the most prevalent (93-100%), but C and D were prevalent too (31-100%). Feral and non treated horse populations showed higher diversity of larval types, being B the more relevant (19-85%). Mann-Whitney test revealed significant differences in the abundance of subpopulations, namely for type A ($p<0,001$), which is more abundant in farm dewormed horses. In Romania the pattern was similar, being Type A the most abundant (59-71%), followed too by types D (11,3-20,3%) and C (16,8-23,7%). Types B and H were found between 0,7-1%. Identification of horse cyathostomin L3 larval stages remains an interesting field of study, v.g. type A with 8 intestinal cells, according to the literature originated by highly prevalent species, v.g., *Cylicocyclus nassatus, Cylicostephanus longibursatus, Cyathostomum catinatum*.

Rezumat

Studiul a fost realizat în perioada 2000-2008, în Portugalia şi România, prin analiza comparativă a prevalenţei şi intensivităţii diferitelor specii de *Cyathostomum sensu latum*, respectiv pe baza caracterelor morfologice ale larvelor de stadiul 3 (L3), provenite din probe de fecale de la cabaline domestice, sălbăticite şi asini. Au fost identificate 8 tipuri morfologice de L3 aparţinând *Cyathostomum sensu lato*: 5 specii cu 8 celule intestinale (A, B, C, D şi G) şi trei cu 6, 7 şi 9 celule (E, F şi H). In Portugalia, intensivitatea cea mai ridicată a înregistrată la *Cyathostomum* tip A (65-98%), urmată de tipul D (3,9-9,7%) şi C (2,3-20,6%). Intensivitatea cea mai redusa a fost înregistrată la larvele de tip B (1,4-2,2%), E, F G şi H (0,3-3,2%). L3 de tip A au avut prevalenţa cea mai ridicată (93-100%), urmate de cele de tip C şi D (31-100%). Populaţiile de cabalinele sălbăticite şi cele nedehelmintizate au prezentat o diversitate ridicată a tipurilor de larve, cele mai frecvente fiind cele aparţinând tipului B (19-85%). Testul Mann-Whitney a scos în evidenţă diferenţe semnificative ale intensivităţii subpopulaţiei de *Cyathostomum sensu lato*, în special pentru tipul A ($p<0,001$), care a fost cel mai frecvent identificat în fermele de cabaline la care s-a aplicat dehelmintizarea. In Romania, situaţia a fost asemănătoare, tipul A având intensivitatea cea mai ridicată (59-71%), urmat de tipul D (11,3-20,3%) şi C (16,8-23,7%). Tipul B şi H a avut o intensivitate de doar 0,7-1%. Identificarea specilor de cyathostome pe baza caracterelor morfologice ale larvelor de stadiul trei rămâne un interesant domeniu de studiu, deoarece conform literaturii de specialitate, larvele aparţinând tipului A cuprind specii cu prevalenţă ridicată: *Cylicocyclus nassatus, Cylicostephanus longibursatus, Cyathostomum catinatum*.

Cuvinte cheie: ecvine, strongili, *Cyathostomum sensu latum*

Key words: equine, strongyls, *Cyathostomum sensu latum*

INTRODUCTION

Cyathostomins are assuming a major importance on horse production as pathogenic agents responsible for unthriftiness, colic, diarrhoea, moderate/high mortality rate and anthelmintic resistance cases.[1,2] The identification of horse strongyles infective larval stages (L3) is extremely important for biological and epidemiological studies but although it is an easy technique for identification there is a lack of information concerning morphological and biological features of cyathostomin L3.[3,4] Horse cyathostomins L3 identification is important since some of them (namely type A with 8 intestinal cells) are originated by prevalent and resistant species (v.g., *Cylicocyclus nassatus, Cylicostephanus longibursatus, Cyathostomum catinatum*)[5,6]. This work presents recent findings on *Cyathostomum sensu latum* (Cyathostomum s.l.) L3 morphotypes together with its application on analysing cyatosthomin populations from feral and domestic horses and donkeys, dewormed and non dewormed, in Portugal and Romania.

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MATERIAL AND METHODS

Phase 1

Random faecal sampling of feral garrons in Peneda-Gerês National Park and individual faecal sampling from domestic horses in Ribatejo, respectively northwest and west centre of Portugal, were performed in July 1999 and January 2000, being collected a total amount of 20 faecal samples/horse group. In Romania faecal samples were collected individualy from 992 domestic equines during 2003-2008 (Fig. 1, 2 and 3).

Glass and Petri dish faecal cultures were performed for 7-14 days according to known techniques.[7,8] Morphometrical parameters were recorded [3,9] and a comparative analysis of *Cyathostomum sensu latum* L3 subpopulations was performed in cultures for every group of horses, aiming the study of prevalence in each horse population studied and their relative abundance or mean intensity, counting different morphotypes in 100 *Cyathostomum s.l.* L3/sample (relative abundance expressed in percentage).

To evaluate differences between *Cyathostomum s.l.* L3 subpopulations among feral and domestic horses in Portugal and abundance differences between portuguese and romanian horses, the non parametric Mann-Whitney test was used, $p<0,05$, with Graph Pad InStat V.3 2003.

Fig. 1 and 2. Feral horse population (northwest Portugal) and domestic population in a horse stud farm (west centre Portugal).

Phase 2.

Faecal samples from domestic horses (3 groups) and donkeys (3 groups) were sampled in different locations in Portugal during epidemiological studies and anthelmintic field trials (2003-2007), to assess differences of *Cyathostomum s.l.* larval subpopulations between dewormed and non dewormed populations of equids as proposed in previous studies.[10,11]

A similar study was performed in Romania and it involved populations of horses, ponies and donkeys as referred above and the relative abundance of the major Cyathostomum morphological types were recorded and compared with the structure of *Cyathostomum s.l.* L3 subpopulations.

Fig. 3. Horses moving to the pasture at Beclean Stud, situated near to the stables.

RESULTS

In phase 1, eight types of *Cyathostomum sensu lato* L3 were recorded in Portugal: five with 8 cells (A, B, C, D and G) and other three not referred in literature, with 6, 7 and 9 cells (E, F and H) (Table 1, Figs. 4 to 7). The majority belonged to *Cyathostomum* A (65-98% abundance), followed by C and D (6-8%). The larval types with lower expression were B and E-H. Type A L3 was the most prevalent one (93-100%), but C and D were prevalent too. Feral horses showed a higher biodiversity of *Cyathostomum s.l.* L3 subpopulations and the comparative analysis with domestic horses showed significant differences ($p<0,01$), namely concerning *Cyathostomum s.l.* type A (Figs. 8 and 9).

In Romania the pattern was similar, being Type A the most prevalent too (59-71%), followed by D (11,3-20,3%) and C (16,8-23,7%). B and E-H were found between 0,7-1%.

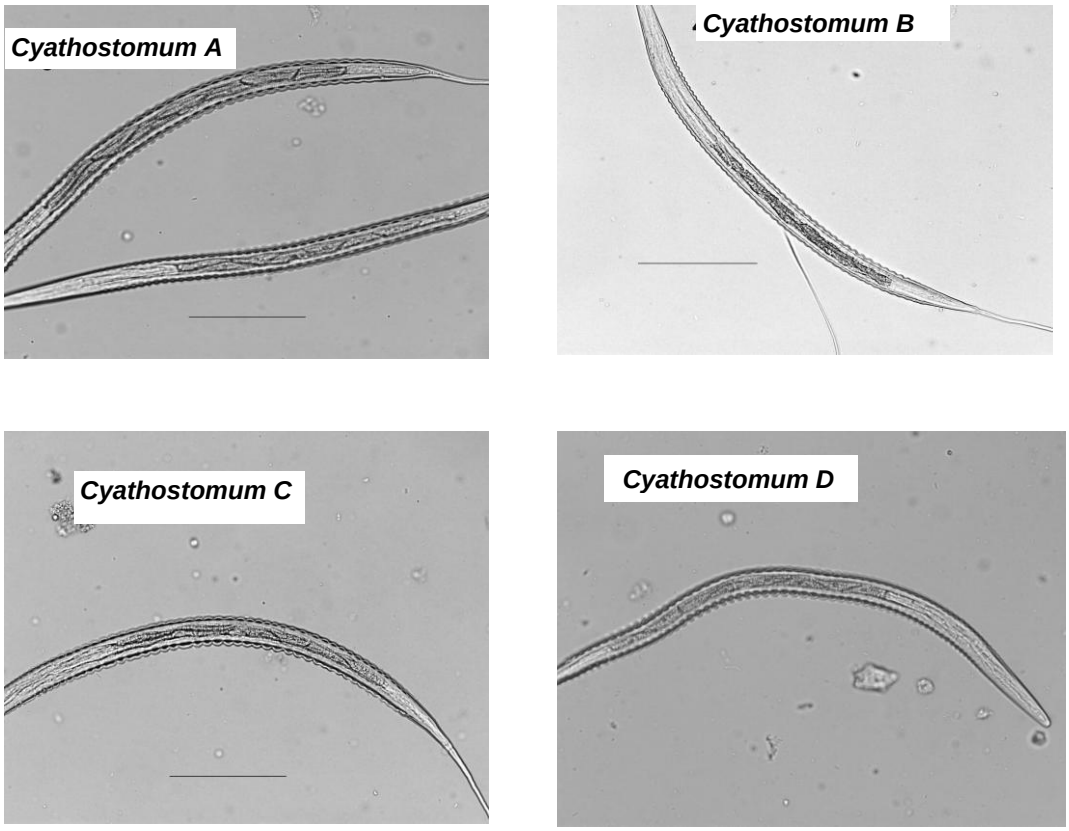
The analysis of average results of the larval subpopulations in horses from both countries showed no significant differences, although the Portuguese results showed a higher shift for the L3 *Cyathostomum* type A (Figs. 10 and 11).

Table 1 - Morphotypes of L3 larval stages of *Cyathostomum s.l.*

L3 Morphotype	Nº intest. cells	Intest. cells arrangement
Cyathostomum A	8	2+6 (first 2 double row, 6 single row)
Cyathostomum B	8	4+4 (8 cells in double row)
Cyathostomum C	8	2+2+4 (4 cells double row and 4 single row)
Cyathostomum D**	8	8 cells in single row (unspecific)

Cyathostomum E**	6	Variable (unspecific)
Cyathostomum F**	7	Variable (unspecific)
Cyathostomum G	8	Variable (unspecific)
Cyathostomum H**	9	Variable (unspecific)

**L3 larval types of *Cyathostomum s.l.* not referred before.



Figs. 4 to 7. Most prevalent *Cyathostomum* s.l. morphotypes: A, B, C and D. 100X . (Originals).

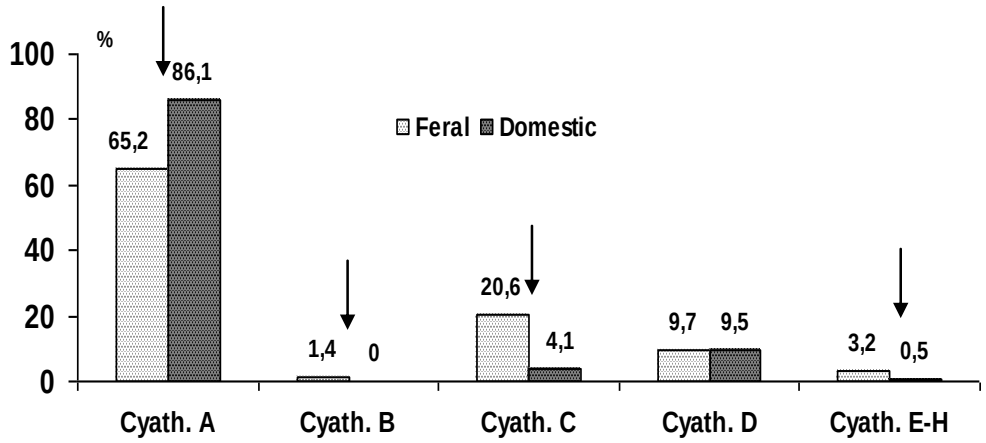


Fig. 8. Differences of relative abundance in domestic and feral horse populations in Portugal (phase 1) (arrows - significant differences, Mann-Whitney test, p<0,001).

In phase 2 domestic and dewormed horses in Portugal had a clear dominance of type A. However, B is more relevant in non treated horses (19-85%), which showed higher diversity of larval types like in the feral horse population. All the other types were found in every group of equids, however the prevalence values were lower in domestic horse populations compared with the feral ones. Concerning relative abundance, the dewormed horses showed higher values of abundance for type A in horses and donkeys and lower values in feral horses. Nevertheless, feral horses showed more representative values for the other types, making this group the one with more variety of larval subpopulations. (Figs.12 to 14).

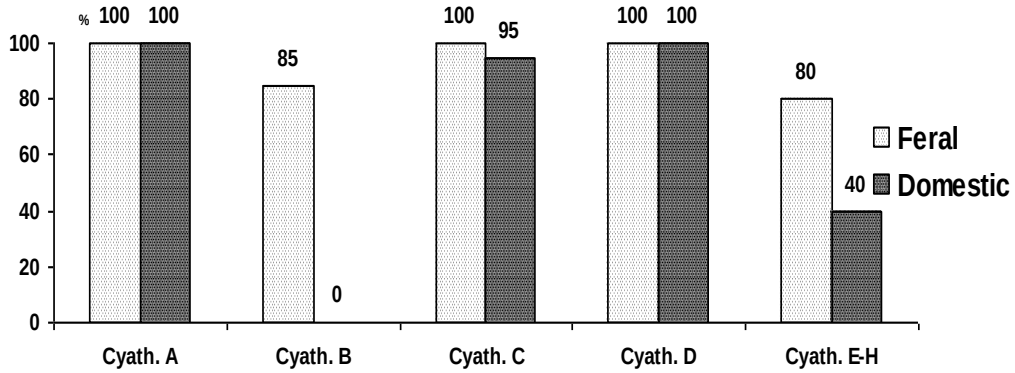


Fig. 9. Differences of prevalence in domestic and feral horse populations in Portugal (phase 1).

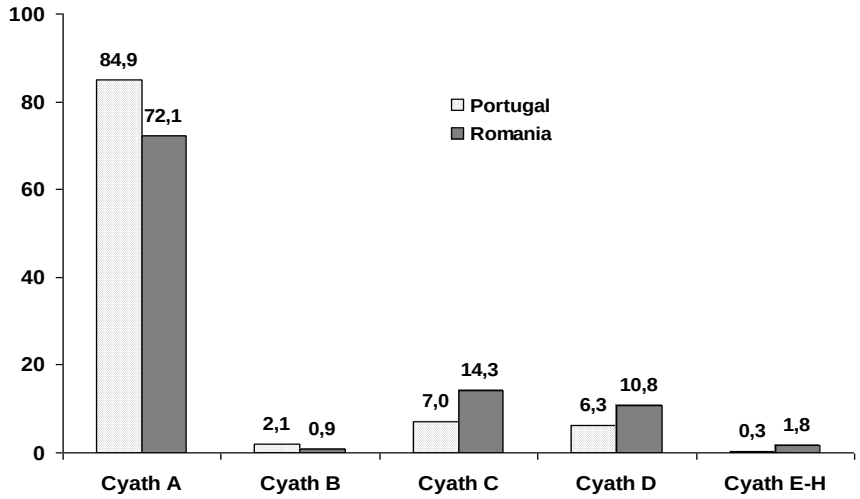


Fig.10. Differences of prevalence of *Cyathostomum s.l.* in horse populations in Portugal and Romania (phase 1).

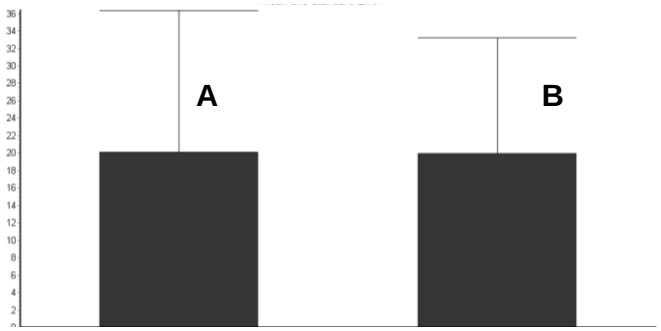


Fig. 11. Median values of relative abundance in horse populations from Portugal (A) and Romania (B) (phase 1). The Mann Whitney test showed a non significant difference between the values of *Cyathostomum* s.l. in these two horse populations (p = 0,84)

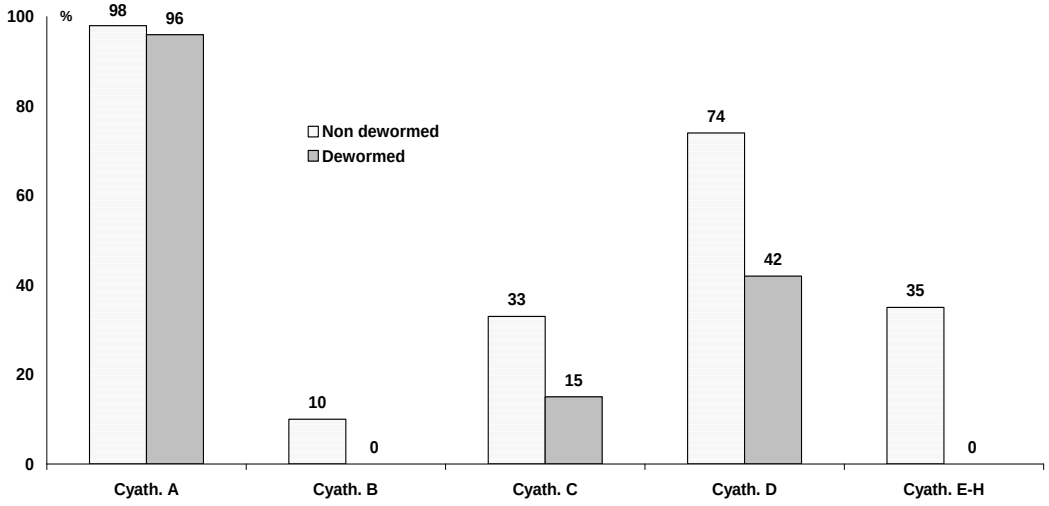


Fig. 12. Differences of prevalence in dewormed (Aveiro B) and non dewormed (Aveiro A) horse populations in Portugal (phase 2).

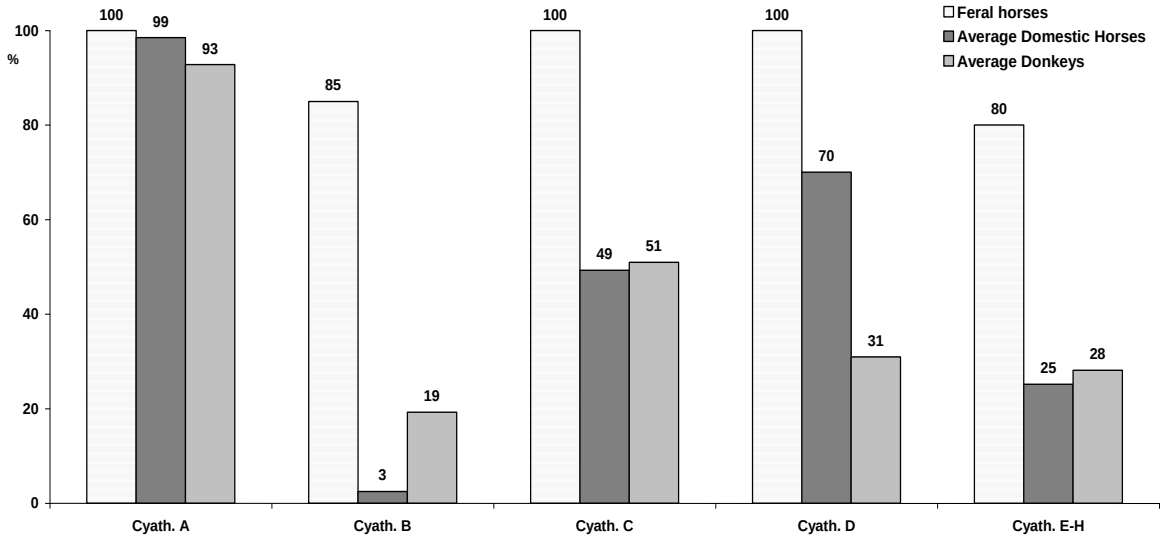


Fig. 13. Differences of prevalence of *Cyathostomum* s.l. in feral and domestic horses and donkey populations in Portugal (phase 2).

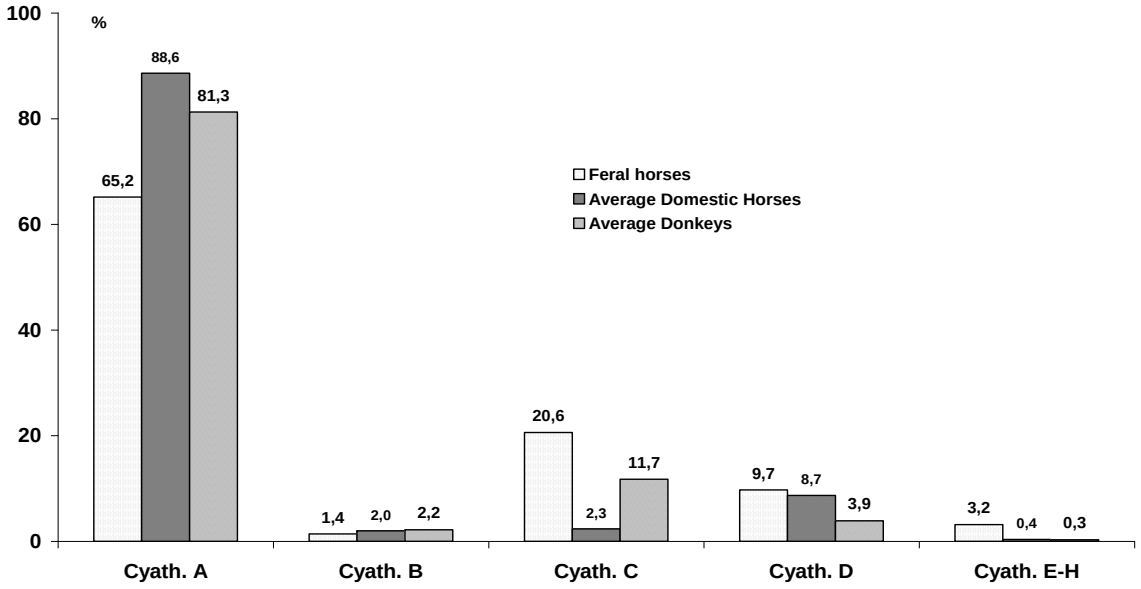


Fig. 14. Differences of abundance of *Cyathostomum* s.l. in feral and domestic horses and donkey populations in Portugal (phase 2).

In Romania, the experiments in phase 2 showed that the global pattern of relative abundance was similar to the one found in Portugal, being *Cyathostomum* type A the most abundant too, followed by types C and D. Nevertheless, A is not so abundant like in Portugal, but consequently types C and D are more abundant, especially in horses and donkeys (Figs. 15 and 16).

The average results concerning the relative abundance in both countries are summarized in the last graphic (fig.16) and we can see that with some differences, the overall pattern is the same and all the differences between the raw values of both countries tested with Mann-Whitney proved to be not significant and followed the results found with the prevalence data (p> 0,05).

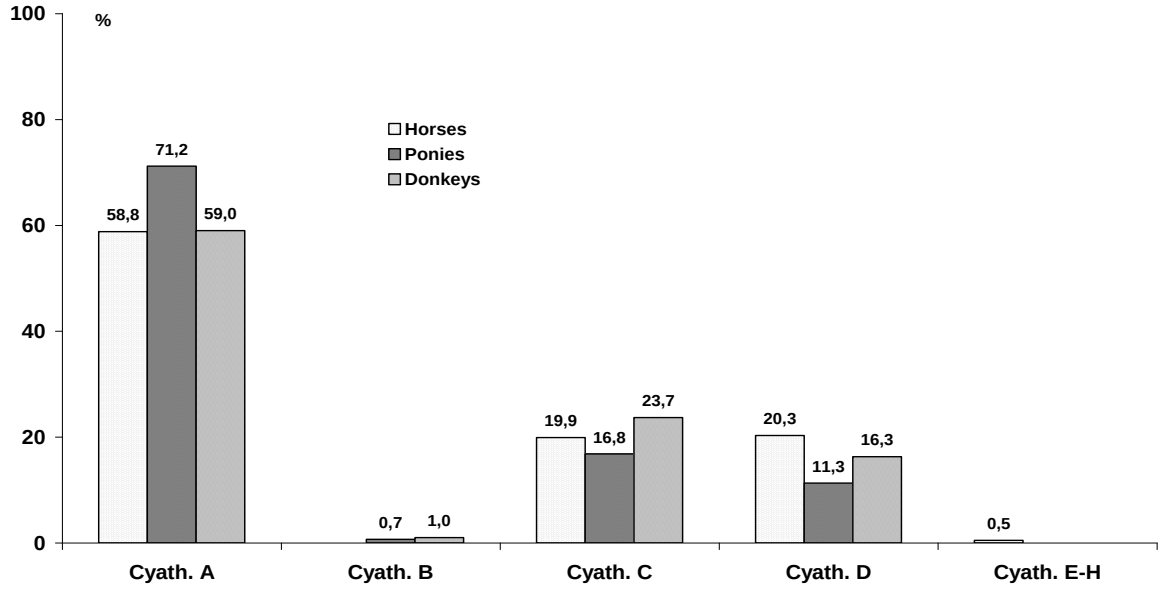


Fig. 15. Differences of abundance of *Cyathostomum* s.l. in horses, ponies and donkey populations in Romania (phase 2).

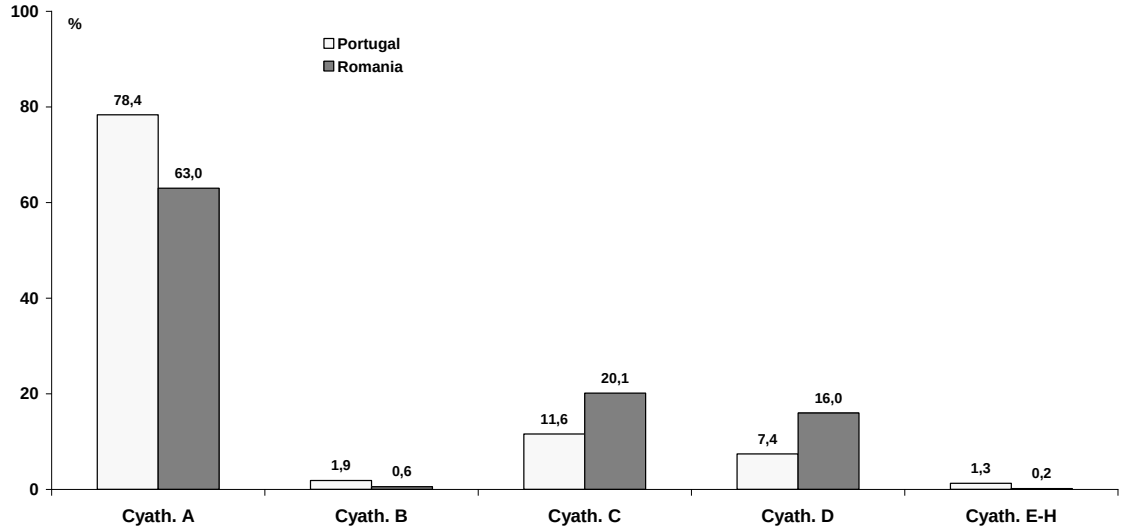


Fig. 16. Differences of relative abundance of *Cyathostomum s.l.* in horse populations in Portugal and Romania (phase 2).

DISCUSSION AND CONCLUSIONS

Based on morphological characters of the *Cyathostomum s.l.* L3 larval stages, there are different subpopulations. The L3 type A (combination 2+6 intestinal cells) is dominant and it may be troublesome since it is generated by the most prevalent and resistant cyathostomins.[2,6,11,12]

There are significant differences between *Cyathostomum* L3 subpopulations in horses regularly dewormed and feral or not dewormed horses. The horse populations with a low frequency deworming program or not submitted to anthelmintic treatments, like feral horses, show a greater diversity of genera and species of Strongyles, being prevalent all the larval types with rates of abundance not so marked like the domestic and dewormed animals. This behaviour of strongyle populations in feral horses can be associated too with the isolation of feral herds and the low number of animals/ha, since they use large pasture areas, preventing a higher rate of infection[13]. This method was used successfully in different horse populations and different geographical locations (Portugal and Romania), with the basic same pattern[12].

This knowledge can have a practical application, since these species belong to the group of cyathostomins with higher abundance and prevalence, contributing for 95% of the total population of small strongyles, being widespread and common in Portugal and Romania as elsewhere[13,14,15].

This method may allow an indirect evaluation of the impact of control measures on cyathostomins diversity, since dewormings may induce an increase of L3 subpopulations related with resistant species of cyathostomins. This way, when we have a shift for type A and a reduced biodiversity of *Cyathostomum* larval types, this may indicate a high deworming pressure on the region, horse farm or animal studied

As major conclusions we may state the following ones:

a) There are different subpopulations of *Cyathostomum s.l.* and type A L3 is dominant, followed by C, D and B;

b) The other morphotypes E to H are residual, but when they are more prevalent and abundant may be associated with horses less dewormed

c) There are significant differences between horses regularly dewormed and rarely or not dewormed, being type A more prevalent and abundant in the first situation;

d) This method allows an indirect evaluation of the status of resistant/susceptible cyathostomin populations, since a reduced biodiversity of larval types may indicate a higher anthelmintic pressure, since the most prevalent and abundant larval types are produced by the most prevalent, abundant and resistant cyathostomin species.

The results presented in this paper must be tested in a larger scale, with more countries and horse populations, so that this pattern can globally indicate a change of horse cyathostomin population towards anthelmintic resistance or not, respectively considering a shift into a narrow or wide biodiversity of L3 larval subpopulations of *Cyathostomum sensu latum*.

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